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(Translated by Ostertag)

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CRICETUS AURATUS W. 1939 AS AN EXPERIMENTAL MODEL IN
ANTHRAX

[Following is the translation of an article by Yu. S. Voronin, S. A. Dzharlygasov, Yu. S. Pisarevskiy and M. M. Faybich, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology), No. 9, 1963, pages 120-125; it was submitted on 21 Aug 1961. Translation performed by Sp/7 Charles T. Ostertag Jr.]

In tests on studying experimental anthrax infection, usually rabbits, guinea pigs and white mice are used (Uchida, 1926; Sobernheim, 1927; Hruska, 1931; Mikhin, 1942; Rozanov, 1952; Shlyakhov, 1960, etc.). Sometimes for this purpose sheep and other domestic animals which are susceptible to the stated infection are used. Apparently susliks are susceptible to anthrax (Tumanskiy, 1948). Spontaneous illnesses with this infection have been noted in gray rats (Mikhin, 1942) and great gerbils (Punskiy and Tsibulevskaya, 1958). Marchette, Lundgren and Smart (1957) studied the susceptibility to anthrax following the intracutaneous infection of 12 species of wild rodents from the state of Utah (USA).

However, in the literature we have not encountered any indications on the susceptibility of golden hamsters (*Cricetus auratus* W. 1939) to anthrax.

Taking into consideration the fact that recently the golden hamsters along with other species of laboratory animals have found wide application during various viral and bacterial experimental infections (Faybich and Dzharlygasov, 1961), we undertook the mission of studying the susceptibility of the animal to anthrax.

In the present tests golden hamsters were used that were 5-6 weeks old and with a weight of 80-120 grams. These animals were picked out of a batch of golden hamsters obtained from the Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR.

Experiments with hamsters do not require special equipment, cages and instruments which are necessary when working with gray rats and rat-like hamsters. It is only necessary to be cautious of bites. The golden hamsters were taken with assayer's tongs [?] by a fold of skin under the lower jaw. The work was performed with an assistant (but it is possible to work without an assistant). Nasal, conjunctival and intracranial infection as well as the taking of blood from the heart was performed under

mild ether narcosis. Under the effect of ether the golden hamsters very rapidly dropped off to sleep in the course of one minute. The effect of the narcosis lasted 2-3 minutes. This time was completely sufficient for all the necessary manipulations. The infected hamsters (2 or 3 each) were maintained in glass jars. Feeding and observation were carried out by the generally accepted rules.

The subcutaneous, peroral, nasal, conjunctival and cutaneous infection of these animals was conditioned by the usual method used in microbiological practice. The experimental animals were infected with a suspension of a spore culture of the causative agent of anthrax, cultivated on nutrient agar in flasks which were maintained in an incubator at 33-34° for four days. At such a regimen of incubation, spore formation reached 98-100%. Before the test the necessary dilutions were prepared according to the optical standards, with a calculation for a specific standard (1:10).

In the tests on the subcutaneous infection, the culture was introduced into the right inguinal region in a volume of 1 ml in dilutions containing 5, 10, 25 and 50 and more spores of the anthrax causative agent. Three hamsters were infected with each dose and as a control the same number of guinea pigs. During this method of infection, all the hamsters as a rule died from 50 spores and more. The death of the animals set in after 18-96 hours (see table). The death of the guinea pigs was noted from the same doses, but in later periods.

For the infection of hamsters through the depilated skin into the parietal region after two days following depilation, 0.1 ml of a solution of spores from an anthrax culture was introduced. In 0.1 ml of the first dilution it contained 100,000, in the second -- 1 million, in the third - 10 million, and in the fourth -- 100 million spores of an anthrax culture. Three golden hamsters were taken for each dilution, and as a control - three guinea pigs for each dilution. Of 12 golden hamsters, 6 died from specific infection, while all the guinea pigs survived.

For infection through scarified skin, 0.1 ml of each of 4 dilutions of spores from an anthrax culture were placed on a depilated sector. Then scarification was carried out through the drop of the applied suspension of spores. In the first dilution, 0.1 ml contained 1,000, in the second - 10,000, in the third - 100,000, and in the fourth - 1 million spores of the anthrax causative agent.

With each dilution 3 hamsters and 3 guinea pigs were infected. In this test the hamsters were infected beginning with a dose equal to 1,000, and the pigs -- with 10,000 spores of the causative agent. In the tests on infection through scarified skin the death of all the hamsters and pigs was observed, beginning with doses equal to 100,000 spores.

The data obtained makes it possible to consider that in comparison with guinea pigs the golden hamsters are more susceptible and sensitive animals to the causative agent of anthrax, especially during subcutaneous and peroral infection.

This gives a basis for recommending hamsters as a very convenient and practicable model for the study of experimental anthrax infection.

In all the dead hamsters, during autopsy there was observed a swelling of tissues in the area where the material was introduced, an insignificant enlarging of the spleen and plethora of the internal organs; a typical anthrax culture was isolated from the blood of all the animals.

We made the most detailed study of the pathologoanatomical picture following subcutaneous infection.

On the second or third day in the area of inoculation of the spores, edema was manifested, reaching full development by the time of the animals' death (figure 1). Macroscopic changes were absent on the skin at the site of introduction of the suspension of spores. The subcutaneous tissue was sharply edematous and saturated with a gelatinous liquid of a yellow color. Edema encompassed the subcutaneous tissue of the abdomen and in the males was spread into the porous tissues of the scrotum. Microscopically in the proper layer of skin and the subcutaneous tissue, a picture was determined of serous inflammation, developing against a background of the sharpest edema. Swollen, weakly staining, connective tissue fibers are separated by edematous liquid and in places are fragmented into individual patches and lumps. The blood vessels are expanded and plethoric, their walls are edematous, the elastic frame is loosened. The cellular reaction in the form of polymorphonuclear leukocytes has a mild nature. The maximum accumulation of leukocytes are concentrated in the papillary layer of the dermis (figure 2). Proliferation of the cells of the porous connective tissue was absent. Individual fibers of muscle bundles lost the transverse striation and acquired a basophilic color. In the lumina of blood and lymph vessels and also among the cellular elements between the connective tissue fibers there was a mass of anthrax bacilli, while at the sites of their greatest accumulation the leukocytic infiltration was considerably weaker. Changes were lacking in the epidermis. In the subcutaneous tissue with foci of accumulation of the causative agent, the leukocytic reaction was weakly expressed (figure 3).

In the lymph nodes a sharp hyperemia of the tissue was detected histologically, and a reduction of follicles due to depletion by lymphoid elements in the lumina -- the anthrax bacilli (figure 4).

In the internal organs macroscopic changes were lacking, apart from a sharply expressed plethora. The spleen was not enlarged or enlarged insignificantly, the pulp was red without scraping. Microscopy of the

internal organs is very interesting. In tissue from the spleen, in its normal structure large numbers of bacilli were determined, filling up the lumina of the sinuses and the intercellular space of the pulp and forming accumulations in the form of foci. In the liver a picture of sharp plethora was noted. The central veins and all the capillaries were flooded with blood. In the lumina of the vessels, thrombi containing the anthrax bacilli were found. The bacilli were also detected in the capillaries and intertissue gaps. Hepatic cells, mainly in the central sectors of the lobules, were found in a condition of granular dystrophy. The kidneys were sharply plethoric, in the interstices of the cerebral layer small foci of hemorrhage were encountered. In addition to the plethora of the glomerules, an accumulation was noted of edematous fluid in Bowman's capsule. The epithelial cells of the tubules were partially in a condition of granular dystrophy. It was possible to see anthrax bacilli in the lumina of the capillaries of the glomerules. In the lungs a disruption of circulation was clearly expressed in the form of a sharp plethora with the presence of foci of hemorrhage and numerous thrombi of minute vessels. The thrombi were made up mainly of fibrin (figure 5). In individual vessels there was a destruction of erythrocytes with the formation of granular masses. All the lung tissue was penetrated by a huge quantity of anthrax bacilli, which, by growing vigorously, filled up the lumina of the alveoli (figure 6).

In other organs and tissues, except for plethora and the presence of the causative agent of the infection, changes were absent.

Thus, the pathologoanatomical changes in hamsters following subcutaneous infection were characterized by the development of a sharp edema of tissues in the area of inoculation, the disruption of circulation inside of internal organs in the form of stagnant plethora and the formation of thrombi, dystrophy of the parenchyma of the liver and kidneys, and finally, primary accumulation of the causative agent in the spleen, lungs, subcutaneous tissue, and the site of inoculation.

Conclusions

1. Golden hamsters are highly susceptible and sensitive to the anthrax causative agent following the subcutaneous, percutaneous, and peroral methods of infection.
2. Hamsters infected with anthrax usually die in the course of 1-3 days. The period of death was found in direct dependence on the dose of microbes introduced.
3. In subcutaneously infected golden hamsters, the local and general morphological changes were identical with the pathologoanatomical picture of anthrax observed in farm and laboratory animals during the same method of infection.
4. Golden hamsters may be utilized along with other species of laboratory animals for the study of experimental anthrax infection.

Literature

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[The following English summary appeared with the Russian article.]

An inquiry was made into the susceptibility and infectious sensitivity of *Cricetus auratus* W., 1939 to *B. anthracis* in subcutaneous, transdermal, oral and intranasal methods of infection. *Cricetus auratus* proved to be more sensitive to this bacillus than guinea pigs. Morphological changes in these animals infected subcutaneously were the same as those in other animals.

Method of infection	Type of animal	Results of infection with various amounts of spores											Aver. Total time of death in hrs)		
		5	10	25	50	500	1,000	5,000	10,000	100,000	1 mil.	10 mil.	100 mil.	in	diel
Subcutaneously	Hamsters Pigs	0/3	1/3	2/3	3/3	3/3	3/3	3/3	-	-	-	-	-	64.5	15/21
Through a depilated sector of skin	Hamsters Pigs									0/3	1/3	2/3	3/3	50	6/12
Through scarified skin	Hamsters Pigs						0/3		2/3	3/3	3/3	3/3		48	8/12
Peroral	Hamsters Pigs						1/3		2/3	3/3	2/3			40	11/12
Nasal	Hamsters Pigs						0/3		0/3	1/3	2/3	1/3		51	0/12
Conjunctival	Hamsters Pigs						0/3		0/3	0/3	0/3	0/3		51	4/12
							0/3		0/3	0/3	0/3	0/3			0/12
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Numerator -- Number of animals died; Denominator -- Number of animals in the test.

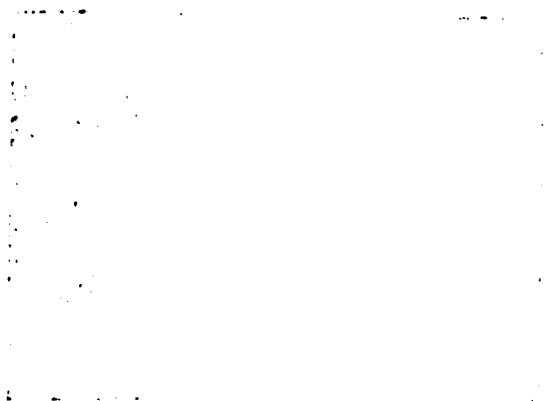


Figure 1. Edema of the soft tissues of the rear extremities, Control on the left.

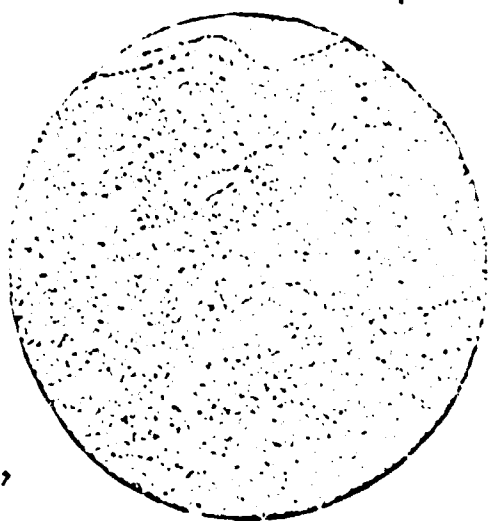


Figure 2. Cutis. Leucocytic infiltration of the dermis.



Figure 3. Subcutaneous cellular tissue. Edema. Solitary leucocytes at the sites of accumulation of anthrax bacilli.

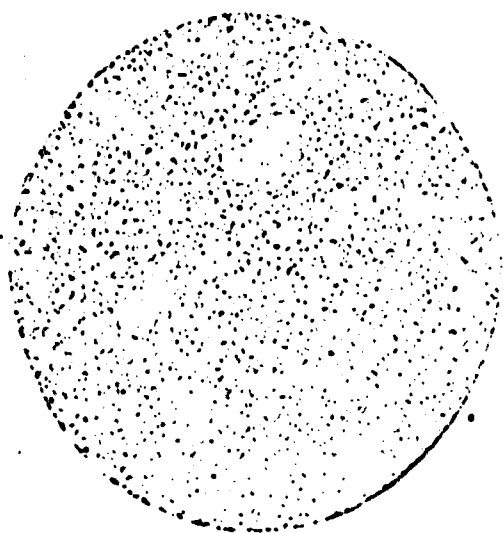


Figure 4. Inguinal lymphatic node. Reduction of follicles. Edema of tissue . Accumulation of bacilli in the lumen of the vessel.

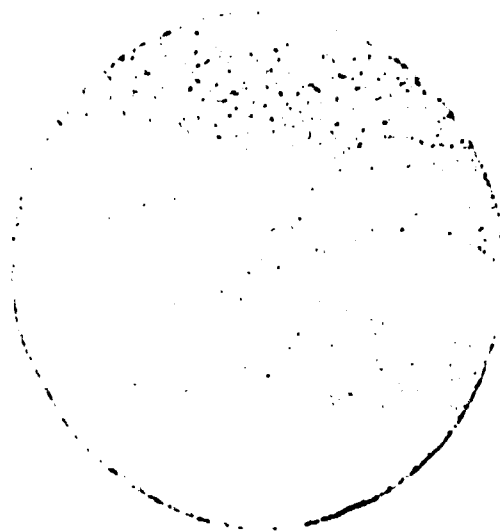


Figure 5. Lung. Thrombus in the lumen of the blood vessel.



Figure 6. Lung. Accumulation of anthrax bacilli in the alveolar walls.